Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 2-29 and 61-67 are pending in the application, with claim 2 being the independent claim. Claims 1 and 30-60 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Applicants reserve the right to pursue the subject matter of the canceled claims in related applications. Claim 2 has been amended to incorporate the recitations of withdrawn claim 1. Claim 6 has been amended to correct the antecedent basis of the claim and further to remove unnecessary language in the claim. Claims 7, 9, 61 and 63 have been amended to make explicit that which was implicit in the claims and the amendments do not narrow the scope of the claim. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Information Disclosure Statement

Applicants believe a paper copy of document number AA3 was previously filed on June 22, 2001 and submit a copy of the stamped postcard forwarding the documents as proof of filing. Applicants submit herein a replacement paper copy of reference AA3, Zupancic *et al.* Applicants respectfully request that this document be considered, and that an initialed copy of the P.T.O. form 1449 citing this reference be returned to Applicants.

Compliance with Sequence Rules

Applicants have amended the Brief Description of the Drawings on pages 10 and 11 of the specification to include the polynucleotide SEQ ID NOs as suggested by the Examiner. Applicants believe that the application is in full compliance with the requirements set forth in 37 C.F.R. § 1.821-1.825. Notification of compliance is respectfully requested.

Objection to the Title

Applicants have amended the title as put forth in an amendment from an International Search Report. Applicants believe that the title is now clear and respectfully request reconsideration and removal of the objection.

Objection to the Abstract

Applicants have amended the abstract to include the isolated polynucleotides for L-lysine biosynthesis of the present invention. Applicants believe that the abstract is now complete and respectfully request reconsideration and removal of the objection.

Objections to the Drawings

Applicants have submitted herein a revised Figure 13 which Applicants believe is now clear. Therefore, Applicants respectfully request reconsideration and removal of the objection.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 22-24, 27-29 and 66 are rejected under 35 U.S.C. § 112, first paragraph, enabling deposit, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skill in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicants have included the date of deposit for deposits NRRL-B30218, NRRL-B30219, NRRL-B30220, NRRL-B30221, NRRL-B30222, NRRL-B30233, NRRL-B30234, NRRL-B30235, NRRL-B30228, NRRL-B30236, and NRRL-B30237. In addition, Applicants have amended the specification to include the deposit information of NRRL-B30359, NRRL-B30628, and NRRL-B30629. Applicants also file herewith a statement certifying that all restrictions on accessibility to said deposits will be irrevocably removed by Applicants upon granting of the patent. Therefore, Applicants respectfully request reconsideration and removal of the rejection.

Claims 8, 9, 16, 25 and 26 are rejected under 35 U.S.C. § 112, first paragraph, written description, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants respectfully traverse this rejection.

Specifically, the Office Action alleges that although a single species of each polypeptide is structurally described in the figures, a description of the common characteristics of each genus, particularly a correlation between the structure and function of these genes is lacking in the specification. It further alleges that without such a correlation, one of skill in the art would be unable to identify other members of each genus

in structural and functional terms. Applicants assert there is ample written descriptive support for the combination of the polynucleotide of SEQ ID NO:2 ***and*** a polynucleotide encoding any one of the polypeptides *asd*, *dapA*, *dapB*, *ddh*, *'lysA*, *lysA* and ORF2. All of the above stated additional polynucleotides are functional in the lysine biosynthetic pathway as demonstrated in Figure 1. In addition, there is literal description for the multi-genic constructs on page 39, lines 1-9. Furthermore, the Examples describe the construction and the efficacy of the multi-genic constructs. Finally, the biological deposit of such constructs is given in the specification at page 43, lines 7-25 demonstrating that Applicants had possession of the invention at the time the application was filed. As stated in M.P.E.P 2163, "Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention." The M.P.E.P. further states that, "An application specification may show actual reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 CFR 1.801 *et seq.*" Therefore, Applicants assert that there is ample written descriptive support for the combination of the polynucleotide of SEQ ID NO:2 and a polynucleotide encoding any one of the polypeptides *asd*, *dapA*, *dapB*, *ddh*, *'lysA*, *lysA* and ORF2. Applicants respectfully request reconsideration and removal of the rejection.

Claims 61, 63-65 and 67 are rejected under 35 U.S.C. § 112, first paragraph, written description, as allegedly containing subject matter which was not described in the

specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Not in acquiescence to the rejection, but rather solely to advance prosecution, Applicants have amended claim 61 to include a functional limitation as suggested by the Examiner. Therefore, Applicants respectfully request reconsideration and removal of the rejection.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 6-15 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the term "said" amino acid biosynthetic pathway genes in claim 6 does not have proper antecedent basis. Applicants have amended claim 6 to now clarify the antecedent basis of the claim. Additionally, it is asserted that the term "amino acid biosynthetic pathway genes" in claim 6 is unclear. Applicants respectfully traverse this rejection.

It is known to those of ordinary skill in the art that genes or gene fragments "directly involved" in the synthesis of amino acids denotes affecting the production of amino acids. In terms of the instant application, these amino acid biosynthetic pathway genes govern the production of the amino acids. One goal of the specification is to increase the synthesis of amino acids and many methods to achieve this are taught, including transforming cells with a polynucleotide thereby increasing the total number of amino acid biosynthetic pathway genes. Moreover, it is asserted that the term "biological activity" is unclear since such a term can encompass enzymatic activity, immunological activity, etc. Applicants respectfully disagree. Taken in the context of the specification, the biological activity of the

polypeptides is that which is involved in amino acid biosynthesis and cannot be viewed as dealing with an immunological activity as suggested in the office action. Therefore, Applicants respectfully request reconsideration of the rejection and further that it be removed.

Claims 7 and 9 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, it is asserted that the term "increased" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. Applicants respectfully traverse this rejection.

At page 25, lines 16-24 the specification provides support for use of the term increased. Methods for screening for increased production of an amino acid are defined as:

> [s]creening for increased production of an amino acid, for example L-lysine, may be determined by directly comparing the amount of L-lysine produced in culture by a *Corynebacterium* species host strain to that of a *Corynebacterium* species transformed host strain in which an amino acid biosynthesis gene or genes are amplified.

Applicants have amended claims 7 and 9 to read on further screening for the detection of the transformed polynucleotide molecule. Therefore, Applicants respectfully request reconsideration of the rejection and further that it be removed.

Claims 8, 9, 16, 25 and 26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicants respectfully traverse this rejection.

Specifically, the Office Action alleges that "'lysA" and "ORF2" are unclear in the absence of a functional definition. As stated in the specification and acknowledged in the Office Action, the function of the other genes in the lysine biosynthetic pathway are given in Figure 1. In addition, the term "'lysA" is defined in the specification on page 5, lines 20-22 which state:

> The term "'*lysA*" refers to a truncated *lysA* gene or amino acid sequence used by Applicants and described *infra*. The term "*lysA*" refers to the full length *lysA* gene or amino acid sequence used by Applicants and described *infra*.

Furthermore, on page 14, lines 6-9, the use of ORF is defined in the specification as:

> Also included are amino acid sequences as encoded by open reading frames (ORF), where the ORF is associated with a lysine biosynthetic pathway operon. These proteins may be identical to those which naturally occur within a host cell and are involved in the synthesis of lysine within that host cell.

Therefore, Applicants believe that the use of 'lysA and ORF2 are clearly defined in the specification and respectfully request that the rejection be reconsidered and further that it be removed.

Claim 63 is rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, it is asserted that the term "the LysA gene" is unclear. Not in acquiescence to the Examiner's rejection but rather to advance prosecution, Applicants have amended claim 63 to read on the encoded polypeptide sequence rather than the LysA gene, as suggested by the Examiner. Therefore, Applicants respectfully request reconsideration and removal of the rejection.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Michele A. Cimbala
Attorney for Applicants
Registration No. 33,851

Date: Sept. 23, 2002

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Version with markings to show changes made

In the Title:

The following Title of the Invention was substituted for the pending Title of the Invention.

Increased Lysine Production by Gene Amplification Using Coryneform Bacteria.

In the Specification

The paragraph beginning at page 1, line 2 was substituted with the following paragraph.

Inventors: Hanke, Paul D.
Li-D'Elia, Lhing-Yew
Rayapati, John
Crafton, Corey M.
Walsh, Holly J.

The paragraph beginning on page 10, line 3 was substituted with the following paragraph.

Figure 1. A schematic of the L-lysine biosynthetic pathway in *Corynebacterium glutamicum* (Sahm *et al.*, *Ann. N. Y. Acad. Sci. 782*: 25-39 (1996)).

The paragraph beginning on page 10, line 6 was substituted with the following paragraph.

Figure 3 A, B. The amino acid sequence of *ask* (ATCC 21529 sequence) (SEQ ID NOS:1-2) ~~(SEQ ID NO:2)~~.

The paragraph beginning on page 10, line 10 was substituted with the following paragraph.

Figure 5 A, B. The amino acid sequence of *asd* (ATCC 21529 sequence) (SEQ ID NOS:3-4) ~~(SEQ ID NO:4)~~.

The paragraph beginning on page 10, line 14 was substituted with the following paragraph.

Figure 7. The amino acid sequence of *dapA* (NRRL-B11474) (SEQ ID NOS:5-6) ~~(SEQ ID NO:6)~~.

The paragraph beginning on page 10, line 18 was substituted with the following paragraph.

Figure 9. The amino acid sequence of *dapB* (NRRL-B11474) (SEQ ID NOS:7-8) ~~(SEQ ID NO:8)~~.

The paragraph beginning on page 10, line 22 was substituted with the following paragraph.

Figure 11 A, B. The amino acid sequence of *ddh* (NRRL-B11474) (SEQ ID NOS:9-10) ~~(SEQ ID NO:10)~~.

The paragraph beginning on page 11, line 3 was substituted with the following paragraph.

Figure 15 A, B, C. The amino acid sequence of full length *lysA* (pRS6) (SEQ ID NOS:13-14) ~~(SEQ ID NO:14)~~.

The paragraph beginning on page 11, line 7 was substituted with the following paragraph.

Figure 17. The amino acid sequence of ORF2 (NRRL-B11474) (SEQ ID NOS:15-16) ~~(SEQ ID NO:16)~~.

The paragraph beginning on page 11, line 9 was substituted with the following paragraph.

Figure 18. A schematic depiction of the construction of the pFC3-KDABHL and pFC3-KDABH'L ~~5 and 6~~ lysine pathway gene constructs of the invention.

The paragraph beginning on page 11, line 11 was substituted with the following paragraph.

Figure 19. Comparison of the aspartokinase (*ask*) amino acid sequence from ATCC13032, N13 and ATCC21529. A consensus sequence of the alignment is depicted and alterations in the coding sequences are shown.

The paragraph beginning on page 11, line 20 was substituted with the following paragraph.

Figure 24. The amino acid sequence of truncated ORF2 (SEQ ID NOS:18-19) ~~(SEQ ID NO:19)~~.

The paragraph beginning on page 11, line 24 was substituted with the following paragraph.

Figure 26. The amino acid sequence of truncated LysA ('LysA)(NRRL-B11474) (SEQ ID NO:21). Underlined L: the last amino acid residue of *lysA* encoded in the truncated product.

The paragraph beginning on page 43, line 7 was substituted with the following paragraph.

Applicants have deposited clones carrying the pK184-KDABH'L multi-gene constructs at an acceptable International Depositary Authority in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The deposits have been made with the Agricultural Research Service, Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604. Deposits made in which the pK184-KDAB or pK184-KDABH'L multi-gene constructs have been integrated into the chromosome of a host cell include the following: (1) the pK184-KDAB plasmid, integrated into the chromosome, deposited as NRRL-B30219 and NRRL -B30221 on September 16, 1999 and (2) the pK184-KDABH'L plasmid, integrated into the chromosome, deposited as NRRL-B30218, NRRL-B30220, and NRRL-B30222 on September 16, 1999. In addition, the pK184-KDABH'L multigene construct in a plasmid configuration, carried in *E. coli* DH5α MCR, was deposited as NRRL-B30228 on September 29, 1999, and the pK184-KDAB isolated plasmid in *E. coli* was deposited as NRRL-B30628 on September 17, 2002. *E. coli* comprising pD11-KDABH'L was deposited as NRRL-B30629 on September 17, 2002. The six gene construct (pDElia2-KDABHL) was deposited

in *E. coli* (NRRL-B30233) on December 16, 1999. *C. glutamicum* comprising pK184-KDABH'L was deposited as NRRL-B30236 on December 16, 1999. *C. glutamicum* comprising pK184-KDABHL was deposited as NRRL-B30237 on December 16, 1999. *C. glutamicum* comprising pDELia2-KDABHP1L was deposited as NRRL-B30359 on October 31, 2000. *Brevibacterium flavum* comprising pDElia2-KDABHL was deposited as NRRL-B30234 on December 16, 1999. *Brevibacterium lactofermentum* comprising pDElia2-KDABHL was deposited as NRRL-B30235 on December 16, 1999.

In the Claims

The following claim 2 was substituted for the pending claim 2.

2. An isolated polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2 ~~claim 1~~.

The following claim 6 was substituted for the pending claim 6.

6. A method comprising:

(a) transforming a *Corynebacterium* species host cell with the polynucleotide molecule of claim 2, wherein said isolated polynucleotide molecule is integrated into said host cell's chromosome ~~thereby increasing the total number of said amino acid biosynthetic pathway genes in said host cell chromosome~~, and

(b) selecting a transformed host cell.

The following claim 7 was substituted for the pending claim 7.

7. The method of claim 6 further comprising screening for said transformed polynucleotide molecule ~~increased amino acid production~~.

The following claim 8 was substituted for the pending claim 8.

8. The method of claim 6 wherein said polynucleotide molecule further comprises at least one of the following:

(a) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *asd* amino acid sequence;

(b) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *dapA* amino acid sequence;

(c) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *dapB* amino acid sequence;

(d) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *ddh* amino acid sequence; ~~and~~

(e) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *'lysA* amino acid sequence;

(f) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *lysA* amino acid sequence; and

(g) a nucleic acid molecule encoding a *Corynebacterium* species ~~*species*~~ lysine pathway *ORF2* amino acid sequence.

The following claim 9 was substituted for the pending claim 9.

9. The method of claim 8 further comprising screening for said transformed polynucleotide molecule ~~increased amino acid production~~.

The following claim 10 was substituted for the pending claim 10.

10. The method of claim 6, wherein said isolated polynucleotide molecule further comprises at least one of the following:

(a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;

(b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;

(c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;

(d) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;

(e) a nucleic acid molecule encoding the *'lysA* amino acid sequence of SEQ ID NO:21;

(f) a nucleic acid molecule encoding the *lysA* amino acid sequence of SEQ ID NO:14; and

(g) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

The following claim 27 was substituted for the pending claim 27.

27. The host cell of claim 26 wherein said host cell is a *Brevibacterium* selected from the group consisting of *Brevibacterium flavum* NRRL-B30218, *Brevibacterium flavum* NRRL-B30219, *Brevibacterium lactofermentum* NRRL-B30220, *Brevibacterium lactofermentum* NRRL-B30221, *Brevibacterium lactofermentum* NRRL-B30222, *Brevibacterium flavum* NRRL-B30234 and *Brevibacterium lactofermentum* NRRL-B30235.

The following claim 61 was substituted for the pending claim 61.

61. The isolated polynucleotide molecule of claim 2 further comprising a promoter sequence where said promoter sequence has at least 95% sequence identity to SEQ ID NO:17, wherein said promoter sequence controls expression of said polynucleotide.

The following claim 63 was substituted for the pending claim 63.

63. The isolated polynucleotide molecule of claim 61 wherein said promoter is operably directly linked to the encoded polypeptide sequence ~~LysA gene~~.

In the Abstract:

The following abstract was substituted for the pending abstract.

The invention provides methods to increase the production of an amino acid from *Corynebacterium* species by way of the amplification of amino acid biosynthetic pathway genes in a host cell chromosome. In a preferred embodiment, the invention provides methods to increase the production of L-lysine in *Corynebacterium glutamicum* by way of the amplification of L-lysine biosynthetic pathway genes in a host cell chromosome. The invention also provides novel processes for the production of an amino acid by way of the

amplification of amino acid biosynthetic pathway genes in a host cell chromosome and/or by increasing promoter strength. In a preferred embodiment, the invention provides processes to increase the production of L-lysine in *Corynebacterium glutamicum* by way of the amplification of L-lysine biosynthetic pathway genes in a host cell chromosome. The invention also provides novel isolated nucleic acid molecules for L-lysine biosynthetic pathway genes of *Corynebacterium glutamicum* such as a threonine-mutated, feedback-sensitive aspartokinase (*ask*), aspartate-semialdehyde dehydrogenase (*asd*), dihydrodipicolinate synthase (*dapA*), dihydrodipicolinate reductase (*dapB*), diaminopimelate dehydrogenase (*ddh*), and diaminopimelate decarboxylase (*lysA*).